## REMARKS

Reconsideration and allowance of the subject application are respectfully requested.

In the above amendments we have revised claims 1, 11, 25, 36, and 52 to specify that the complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis so as to effect lipopolysaccharide O-sidechain synthesis in vivo. Support for this is found in the specification at page 2, line 28 – page 3, line 13; page 4, line 29 – page 5, line 16; and page 6, lines 21-26. Claims 1, 22, 25 and 36 are further amended to specify (1) that the *Brucella* host cell contains at least two mutations so as to effect sufficient attenuation and (2) that the association between the *Brucella* host cell and the DNA construct is such that following exposure to a mammal the DNA construct gradually separates from the *Brucella* host cell, whereupon the *Brucella* host cell reverts to a rough phenotype that is rapidly and safely cleared from the mammal. These revisions are fully supported by the disclosure, for instance at page 3, lines 6-16, page 4, lines 8-11, page 9, lines 13-21, page 15, lines 24-27, and the Examples section, as well as other parts of the disclosure.

Claims 19, 44 and 54 are amended to cancel the phrase "and genes encoding therapeutic molecules or enzymes producing therapeutic molecules" in response to the Examiner's objection under 35 U.S.C. §112, first paragraph.

Claims 6, 7, 10, 16, 17, 22, 24, 30, 31, 34, 41, 42, 48, 50, 51, 57, and 59-68 are canceled without prejudice.

New claims 70-77 are added which depend from claim 1, 11, 25 or 36, and further require that the DNA construct would be cleared out from a mammal in about eight weeks or less, or that the *Brucella* host cell contains three mutations. Support for these amendments is found in the specification at page 4, lines 9-11, page 13, line 29 -page 14, line 1, page 17, lines 9-11, page 19, lines 28-29, page 20, lines 23-24, page 9, lines 13-21, and Figures 5, 7, 8 and 9, as well as the Examples section.

No new matter is introduced by these amendments to the claims, or by new claims 70-77, and entry and full consideration are requested. With entry of this Amendment claims 1-5, 8-15, 18-21, 23, 25-29, 32-33, 35-40, 43-47, 49, 52-56, 58, 69 and 70-77 will be pending.

On page 2 of the Office Action, the claims are objected to for several formalities. In response, we have added the appropriate punctuation of a period at the end of claims 1, 30 and 31, and have corrected the issued of the antecedent basis for the phrase "the immunogenic composition" in claims 35, 45, 46, 49, 55 and 56.

On pages 2-4, claims 1-58 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention at the time the application was filed. The Examiner is objecting to four separate phases in the claims. Specifically, one phrase is in claims 1, 11, 25, 36, 50 and 52: " . . . a complementation DNA fragment which is operably linked to the promoter and which complements a rough conferring mutation". The Examiner stated that, because of this phrase, the scope of the claims includes numerous structural variants, and further that the disclosure fails to describe the common attributes or characteristics that identify members of the genus that is highly variant. In response, we have amended independent claims 1, 11, 25, 36, and 52 (claim 50 is canceled) to specify that the complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis so as to effect lipopolysaccharide O-sidechain synthesis in vivo. This revision provides structural metes and bounds to the DNA fragment by limiting the scope to a narrow class of peptides. Such class of peptides are well known in the art – as noted in the specification at page 2, "a wide array of genes integral to lipopolysaccharide biosynthesis may also be used [as complementation DNA]." Also, at page 3 it is stated:

> More preferably, [the complementation DNA fragment] encodes a peptide including a lipopolysaccharide O-sidechain, so that when the vaccine is administered to a vaccinee, the lipopolysaccharide O-sidechain peptide is produced in vivo and an antibody to the lipopolysaccharide O-sidechain peptide is produced by the vaccinee in response.

Our specification describes the wboA gene as the preferred complementation DNA fragment, but as is known in the art there are other genes that are also integral to lipopolysaccharide O-side chain biosynthesis. Following the guidance in our specification, these genes could be mutated using procedures known in the art to remove the *Brucella* LPS O-sidechain and thus make a rough phenotype. For instance:

| Gene | Product | Putative Function |
|---|---|---|
| gmd | Gmd | GDP-mannose dehydratase |
| per | Per | Perosamine synthetase |
| pgm | Pgm | Phosphoglucomutase |
| pmm | Pmm | Phosphomannomutase |
| $manB_{OAg}$ | ManB | Mannose isomerase |
| $manC_{OAg}$ | ManC | Mannose guanylyltransferase |
| wzm | Wzm | O-antigen export permease |
| wzt | Wzt | ATP-binding protein |
| wbkB | WbkB | no similarity to known genes |
| wbkC | WbkC | Methionyl tRNA formyltransferase |
| wbkA | WbkA | N-formyl-perosaminyltransferase |
| wboA | WboA | mannosyltransferase, glycosyltransferase |

LPS core synthesis genes:

| | | |
|---|---|---|
| $manB_{core}$ | ManB | Mannose isomerase |
| $manC_{core}$ | ManC | Mannose guanylyltransferase |

It is known that if these genes are removed or altered, the *Brucella* strain will consequently be rough. Evidence of this is found in the prior art references (1) Cloeckaert A, Grayon M, Verger JM, Letesson JJ, Godfroid F. Conservation of seven genes involved in the biosynthesis of the lipopolysaccharide O side chain in Brucella spp. Res Microbiol. 2000 Apr;151(3):209-16; and (2) Godfroid F, Cloeckaert A, Taminiau B, Danese I, Tibor A, de Bolle X, Mertens P, Letesson JJ. Genetic organisation of the lipopolysaccharide O-antigen biosynthesis region of brucella melitensis 16M (wbk). Res Microbiol. 2000 Oct;151(8):655-68. Both are published before our priority date of December 11, 2003. These references and other relevant ones are cited in the attached PTO-1449 Form.

It is submitted that the claims now recite our invention in a way that meets the criteria of 35 U.S.C. §112, first paragraph. Withdrawal of the rejection of these claims is therefore requested.

The second phrase objected to by the Examiner is in claims 10, 24, 34 and 48: ". . . the complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis". These claims are canceled, so this objection is believed moot. However, similar (but not exact) language is now included in independent claims 1, 11, 25, 36, 50 and 52. This language is supported by the specification and by what was known in the art at the time our application was filed, as explained in the preceding paragraph and documented by the attached references. Withdrawal of the rejection of these claims is therefore requested.

The third phrase is in claim 22: " . . . [the complementation DNA fragment] encodes an enzyme synthesized lipids and/or polysaccharides". We have canceled this claim above without prejudice or disclaimer, and withdrawal of the rejection of this claim is therefore requested.

The fourth phrase is in claims 19, 44 and 54: ". . . genes encoding thereapeutic molecules or enzymes producing therapeutic molecules". We have canceled this language from these claims, and withdrawal of the rejection of these claims is therefore requested.

On pages 4-6, claims 25-49 (presumably claims 35, 45, 46 and 49 should have been included) are rejected under 35 U.S.C. §112, first paragraph on the grounds that the specification does not adequately support our vaccine claims. The Examiner is contending that there is not enough data in the specification to show that the claimed vaccines would be effective.

We first note that page 18, line 3 – page 24, line 3 provides a reasonably detailed explanation of the vaccine embodiment of our invention. Production of WRRP1 and other strains are detailed. As described, WRRP1 has the same protective abilities of vaccine strain WR201. A prototype was developed of a live, attenuated *B. melitensis* vaccine strain that expresses protective antigens from three known threat agents—it was tested it for safety, immunogenicity and protective efficacy in appropriate animal models.

Description of how to insert heterologous antigens is discussed. Dosage regimens for humans are disclosed. Guidance for successful production, feasibility of using live bacterial vectors for immunization against heterologous antigens, appropriate promoters, survival of the vaccine strain in vivo and preferred strains for increasing protective immunity are disclosed. (Also see Examples 3, 4 and 6)

> It is well settled that patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art. However, there must be sufficient disclosure, either through illustrative example or terminology, to teach those of ordinary skill how to make and use the invention as broadly as it is claimed. Enzo Biochem, Inc. v. Calgene, Inc., 188 F. 3d 1362, 1138 (Fed.Cir. 1999) (quoting In re Vaeck, 947 F.2d. 488, 496 & n.23 (Fed.Cir. 1991)).

Our disclosure details a reasonable quantity of experimentation necessary to practice the invention, a reasonable amount of guidance how to make and use the invention, and working examples. Our claims as amended have revised the breadth of what is covered, clearly within what is described in detail. The methods needed to practice the invention were well known at the time of the invention, and there was a high level of skill in the art at the time the application was filed. Our disclosure describes the entire procedure for making at least one vaccine that falls within the scope of the claims. This is believed sufficient to meet the requirements of an enabling vaccine claim. In re Wands 858 F. 2d 731 (Fed.Cir. 1988).

Second, we have amended the vaccine claims to narrow the scope, which brings the claims well within what is described in the specification. Reconsideration is requested.

On pages 6-8, claims 4-7, 14-17, 28-31, 39-42 and 69 are rejected under 35 U.S.C. §112, first paragraph, on the ground that the specification lacks complete deposit information for the deposits of ATCC accession numbers PTA-3753 and PTA-3754 and for the plasmid pGSG5. As for PTA-3753 (WRRP1) and PTA-3754 (WRR51), these host cells have been deposited on October 3, 2001 with the American Type Culture Collection (ATCC). Our understanding is that the deposit was made according to the

terms of the Budapest Treaty; however, the official deposit receipt cannot be located, and we are in contact with the ATCC to get a copy of the deposit receipt. As soon as we get confirmation we will submit the requisite statement that the host cells are known and publicly available, etc., or if necessary we will cancel the claims. Regarding the plasmid pGSG5, this strain was not deposited with the ATCC. It is believed that depositing the strain is not necessary given the detailed description in the specification, especially in Figure 10. Figure 10 shows plasmid pGSG5 in detail in a graphic map, describing specific restriction enzyme recognition sites. "pGSG5-9" designates the names of sister clones confirmed in the creation of the construct. Page 14, lines 8-10 confirm that Figure 10 is a map of pGSG5. Someone having ordinary skill in this art would be enabled to make and use pGSG5 by the sufficiently detailed description in our disclosure. Reconsideration of the objection to pGSG5 is requested.

On pages 8-12, the Examiner has lodged three art rejections -- all are based on the inventor's earlier U.S. Patent 6,444,445 and its international counterpart. Specifically, claims 1-58 are rejected under 35 U.S.C. §102(a) and §102(e) as anticipated by, and under 35 U.S.C. §103(a) as obvious over, U.S. Patent 6,444,445; and are also rejected under 35 U.S.C. §102(b) as anticipated by, and under 35 U.S.C. §103(a) as obvious over, the corresponding international application WO 99/37783.

The Examiner's reasoning for all of these rejections is the same: he states that the earlier '445 patent and international counterpart WO 99/37783 both mention that the rough-phenotype single-deletion mutant WRR51 was restored to smooth phenotype via a plasmid construct containing a synthetic copy of the rfbU (wboA) gene. The Examiner is equating this to the claimed complementation DNA fragment that effects a smooth phenotype in a rough phenotype Brucella host cell.

What the '445 patent and its international counterpart do not mention is that the experiment with single-deletion mutant WRR51 gave poor results when ultimately tested (data not shown in the '445 patent or international counterpart). It turned out that the single-deletion mutant was insufficiently attenuated when combined with the DNA complementation construct—it persisted too short a time in the mammal for it to be reasonably effective. The double-mutant WRRP1 had its own problems that had to be

worked out – until the inventors actually did empirical work with the WRRP1 they did not know what effect it would have in a mammal. They had originally desired the double-mutant *Brucella* host cell to be stable (not decay over time) because they believed that would be necessary to deliver heterologous antigens to the mammal. It was a surprise that a double-mutated host cell when combined with the DNA construct decayed slowly over time (i.e., within about 8 weeks), reverting the host cell back to a smooth phenotype that can be safely cleared from a mammal. This could not have been predicted from anything in the '445 patent or its international counterpart. This feature – which is now affirmatively recited in all independent claims – gives our invention an important safety advantage over all existing *Brucella* vaccines. (See pages 15-16 in our specification.)

To clarify our invention, we have amended independent claims 1, 11, 25 and 36 to specify new limitations that are believed to distinguish over the '445 patent and WO 99/37783: (1) the *Brucella* contains at least two mutations so as to effect sufficient attenuation, (2) the complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis so as to effect lipopolysaccharide O-sidechain synthesis in vivo; and (3) the association between the *Brucella* host cell and the DNA construct is such that following exposure to a mammal the DNA construct gradually separates from the *Brucella* host cell, whereupon the *Brucella* host cell reverts to a rough phenotype that is rapidly and safely cleared from the mammal.

The cited references do not disclose a double-mutated *Brucella* host cell coupled with a plasmid containing complementation DNA fragment encoding a peptide required for lipopolysaccharide O-sidechain synthesis, where the association between the *Brucella* host cell and the DNA construct is such that following exposure to a mammal the DNA construct gradually separates from the *Brucella* host cell, and consequently the *Brucella* host cell reverts to a rough phenotype that is rapidly and safely cleared from the mammal. This was not discussed in the '445 patent or WO 99/37783 – the use of a plasmid construct was only in connection with single-mutant WRR51 and nothing further was suggested. The associated discussion in the '445 patent at column 2, lines 62-66 focused on the inability of the plasmid construct to restore the smooth phenotype to the VTRA1

transposon mutant. Certainly, the gradual clearing of the double-mutant from the mammal was not suggested or even considered.

In summary, the two patent documents cited by the Examiner do not teach, make obvious, or suggest any compositions that could be used to provide the properties of compositions and serve the uses of compositions presently claimed. Reconsideration is requested.

On pages 12-13, claims 1-58 are rejected on the ground of obviousness-type double patenting as unpatentable over claims 1-13 of U.S. Patent 6,444,445. We request that this rejection be held in abeyance until the other issues are resolved.

On page 13, claims 25-49 (vaccine claims) are objected to under 37 C.F.R as being substantial duplicates of claims 1-24 (immunogenic composition claims). We first note that no claims are found yet allowable, and thus this objection is premature. Secondly, it is accepted practice for biotech patents to include both immunogenic composition claims and vaccine claims, even though they be quite related. In addition, court decisions have confirmed applicant's right to restate (i.e., by plural claiming) the invention in a reasonable number of ways. Indeed, a mere difference in scope between claims has been held to be enough. In this case, our specification makes clear that immunogenic compositions and vaccines are separate embodiments—restatements of the invention in different ways. Their differences are well-understood in the art; for instance, vaccines are designed and used for one main purpose: upon administration a vaccine stimulates antibody production or cellular immunity against the pathogen but is incapable of causing severe infection. An immunogenic composition, while having similar properties of immunogenicity as a vaccine, need not rise to the level of stimulating immunity against a disease, and can have other uses such as research, diagnostics, etc. The vaccine claims and immunogenic composition claims are not the same in scope, and there is more to each type than just a mere difference in wording. Reconsideration is requested.

With the January 23, 2007 Office Action, the Examiner returned our PTO-1449 forms, with all citations initialed and dated – except three: (1) Elberg, "Immunity to Brucella Infection", Vol. 53, No. 4, Medicine, pages 339-356 (1973); (2) Sadoff et al.,

"Oral Salmonella typhimurium Vaccine Expressing Circumsporozoite Protein Protects Against Malaria", Science, Vo. 240, pages 336-338 (15 April 1988); (3) Pappagianis et al., "Immunization Against Brucella Infections. Effects of Graded Doses of Viable Attenuated Brucella Melitensis in Humans", American Journal of Epidemiology, Vol. 84, No.1, pages 21-31 (1966). The Examiner noted that the PTO-1449 form citations for these references did not include publication dates. To address this, we submit here another PTO-1449 Form that includes these reference citations complete with publication dates. Per the suggestion of the Examiner during a telephone call in February 2007, we are not including additional copies of these references since copies were submitted with the original PTO-1449 form filed September 15, 2005.

All of the Examiner's outstanding art rejections have been addressed, and the application is believed to be in allowable form. Notice to that effect is earnestly solicited. If the Examiner has any questions or would like to make suggestions as to claim language, she is encouraged to contact Marlana K. Titus at (301) 977-7227.

Respectfully submitted,

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